

WHAT IS CLAIMED IS:

- 5 1. A substantially pure Type IIG restriction endonuclease obtainable from *Citrobacter* species 2144 (NEB#1398) (ATCC Patent Accession No. PTA-5846) or from *Escherichia coli* NEB#1554 (ATCC Patent Accession No. PTA-5887).
- 10 2. An isolated DNA obtainable from *Escherichia coli* NEB#1554 (ATCC Patent Accession No. PTA-5887) or from *Citrobacter* species 2144 (NEB#1398) (ATCC Patent Accession No. PTA-5846).
- 15 3. Isolated DNA encoding the restriction endonuclease of claim 1, wherein the DNA comprises a first DNA segment expressing an endonuclease and methyl transferase catalytic function and a second DNA segment encoding a sequence specificity function of the restriction endonuclease wherein the
- 20 first and second DNA segments comprise one or more DNA molecules.
- 25 4. A substantially pure restriction endonuclease according to claim 1 capable of recognizing at least one sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35, and cleaving the DNA on both sides of the recognition sequence.

5. A recombinant DNA vector, comprising: at least one of a first DNA segment coding for the restriction and modification domains of CspCI restriction endonuclease and a second segment coding for the specificity domain of the restriction endonuclease.

6. A host cell transformed with a first DNA segment coding for the restriction and modification domains of CspCI restriction endonuclease and a second segment coding for the specificity domain of the restriction endonuclease wherein the first DNA segment and the second DNA segment are contained within one or more DNA vectors.

7. A method for obtaining the endonuclease of claim 1, comprising cultivating a sample of *Citrobacter* species 2144 (NEB#1398) or a host cell according to claim 6 under conditions favoring the production of the endonuclease; and purifying the endonuclease therefrom.

8. A method of making a Type II restriction endonuclease having an altered specificity; comprising:

(a) selecting a restriction endonuclease from a set of enzymes wherein each enzyme in the set is characterized by a modular structure having a specificity subunit and a catalytic subunit, the specificity subunit further comprising an N-terminal domain for binding one half site of a bipartite recognition

sequence and a C-terminal domain for binding a second half site of the bipartite recognition sequence;

(b) modifying the specificity subunit; and

(c) obtaining the Type II restriction endonuclease with altered specificity.

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9. A method according to claim 8, wherein modifying the specificity subunit in step (b) further comprises substituting the N-terminal domain with a second C-terminal domain or substituting the C-terminal domain with a second N-terminal domain.

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10. A method according to claim 8, wherein modifying the specificity subunit further comprises substituting the N-terminal domain or the C-terminal domain or both N-terminal and C-terminal domain with a binding domain from a second restriction endonuclease or methyltransferase.

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11. A method according to claim 8, wherein modifying the specificity subunit further comprises mutating the N-terminal domain, the C-terminal domain or both domains to alter the binding specificity.

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12. A method according to claim 8, 9, 10 or 11 wherein modifying the specificity subunit further comprises changing the length of the spacer amino acid sequence between the N-terminal and C-terminal domains of the specificity module.

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13. A method according to claim 10, wherein the second restriction endonuclease or methyltransferase is selected from a group consisting of a Type I restriction endonuclease, a Type IIG restriction endonuclease and a γ -type m⁶A methyltransferase.

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14. A method according to claim 8, wherein the specificity subunit and the catalytic subunit are encoded by different genes.

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